HAEMOPHILIA TREATMENT IN DEVELOPING COUNTRIES. A SIMPLE METHOD FOR THE PRODUCTION AND PRESERVATION OF ENRICHED CRYOPRECIPITATE.

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INTRODUCTION

In 1980 at the 1st International Haemophilia conference in Bonn, it was unanimously accepted that treatment should be accessible to all haemophilia patients on an equitable basis. It was also accepted that a minimum of 20,000 units should be made availabe to each patient per year so as to prevent major bleeding episodes, minimize chronic crippling and improve the overall quality of life of the haemophiliac[1].

Today, in spite of all efforts to realise these fundamental expectations there seems to be a growing discrepancy in availability, enlarging the gap in equitability between the industrialized and the developing world. There is not without reason increasing excitement about the development of sophisticated purification technology, using immunoadsorption principles and the progress made in rDNA production of antihaemophilia factor.
However, we should realize that for a large group of haemophilia patients, born in and bound to developing countries, there is still no major progress noticeable. Even the availability of the prime commodity, human plasma, stands in the way of equitability in haemophilia treatment. Conventional concentrates are only accessable for the happy few, who can afford the costs and above all live in an area where supply is reasonably constant and guaranteed. In most developing countries there are many other and more important priorities than haemophilia

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care. The blood transfusion system is still in the more primitive generation stage and blood donation often a source of income, wellcomed by those who preferably should not share their blood with others.

To ultimately reach the goals as stated and accepted in Bonn 1980, a fundamental structuring of the blood supply through a Government supported, society oriented blood transfusion organisation is needed[2]. Optimal use of the blood donated in a fully accepted and clinically practised component policy will provide the necessary availability of both source material and therapeutic components[3].
For haemophilia care, plasma as a source material and cryoprecipitate as the principal therapeutic component should be on the list of each national blood transfusion programme.

In most developing areas, climate, transport facilities, distances to cover, poorly developed primary health care systems and more primitive living environments further hamper adequate and equitable haemophilia care.
In designing a simple, reliable, reproduceable and easy to handle treatment modality for haemophilia the following requirements have to be met:
1. A blood transfusion service equipped according to the principles of a second generation facility - multiple bloodbag system, refrigerated centrifuge capacity, freezing and thawing capacity, freeze drying capacity.
2. Clinical blood transfusion practise based on component policy.
3. Dedicated and supported leadership.

We looked into the possibility to produce a cryoprecipitate with simple and reliable technology, freeze dried and virus inactivated; it is easy to handle under difficult circumstances of climate, transport over distances, storage and use in a more primitive environment.

MATERIALS AND METHODS

Procedure
– Blood is collected in citrated (CPDA-1) double or triple blood bags, centrifuged at 4°C sufficiently long and hard to recover cellfree plasma. The plasma is snap frozen at –50°C in alcohol dry-ice. The alcohol is stored cold in a

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standard bloodbank freezer to reduce dry-ice consumption.

- The snap frozen plasma is thawed in a 4°C waterbath (water and ice cubes), either rocking or with rotating water until the frozen plasma has turned into a slushy mass.
  The bags are then centrifuged at 4°C in precooled buckets, sufficiently long and hard to spin the cryoprecipitate optimally to the bottom of the bags.
- After removing the supernatant plasma, (satellite bag), the cryoprecipitate is allowed to redissolve at 37°C, pooled under aseptic precaution to larger units (i.e. 4 donor units) and stored frozen at –30°C or lower.
- When sufficient numbers of cryo-pools are thus collected, they are rethawed at 4°C under the same conditions as before, pooled under aseptic precautions to larger units of 40 donations (10 x 4 units) centrifuged at 4°C, sufficiently long and hard to allow separation of the cryoprecipitate mass from the supernatant. The supernatant is decanted and discarded.
- The second cryoprecipitate is redissolved at 37°C in 200 ml protectant buffer (pH 6.5) and filtered at 80 and 20 um.
- The final cryoprecipitate is then filled at 40 ml allquots in 100 ml sterile freeze drying bottle, labelled and snap frozen at –50°C for freeze drying and subsequent heat treatment of the dried material in a laboratory oven at 60°C for 24–72 hours.
- The vials with dried finished product can be stored at temperatures of 1–8°C for up to 2 years. For therapeutic use the dried material easily dissolves in half the volume of water for injection, reducing the volume to be injected to 20 ml and increasing the Factor VIII concentration per vial.

Bloodbag systems

Depending upon availability and subsequent clinical use of the red cells and cryosupernatant plasma a double or a triple bloodbag system may be used.

1. Double bags - recovers modified whole blood and cryoprecipitate.
2. Triple bags - recovers red cell concentrate, cryoprecipitate and cryosupernatant plasma for clinical application or fractionation.

Protectant buffer

To protect the labile Factor VIII complex molecule from deterioration during freeze drying and heat treatment a protectant buffer is used, which does not interfere with inactivation of the lipid enveloped HIV by heat.

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formula:	
aminoacids solution	7.5 g/l
glycine	12.5 g/l
Na_3 citrate $2H_20$	3.3 g/l
pH	6.5

as source of aminoacids any commercial or local hospital pharmacy multiaminoacids solution is suitable.[4]

Laboratory controls

Blood donations are routinely controlled for ABO and RhD grouping, irregular antibodies, HBsAg, anti HIV and treponema antibodies. Following freeze drying and heat treatment the batches produced are controlled for FVIII:C, total protein and fibrinogen content, sterility and pyrogen, and additional testing of HBsAg and anti HIV.

Upon completion of all tests and acceptance of results final labelling and release from quarantine is done.

During the development of the method, batches were assayed for FVIII RRCo, FVIII RAg and vWF multimers, as well as FXIII (FSF) content. For practical and logistical reasons the recovery of FVIII:C, total protein and fibrinogen from the original cryoprecipitate stored for 11 and 15 months respectively, as compared to short term (3 wk) storage at −40°C was assessed.

RESULTS

In contrast to the yellow appearance of freeze dried routinely made cryoprecipitate, the product is a snow white powder, easily dissolving within 10 minutes at room temperature to a slightly opalescent solution. The characteristics are given in table 1.

FVIII:C	IU/ml	10.6	tot. prot. g/l	44.4
FVIII RRCo	IU/ml	14.7	fibrinogen g/l	32.0
FVIII RAg	IU/ml	11.0	anti-A titer	2
vWf multimers		normal	anti-B titer	2
Specific activity		0.2	FXIII IU/ml	10.0

table 1

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The product (SRBG-D) compares favourably to the commercially available Cryo 250, a freeze-dried preparation produced by the Central Laboratory of the Dutch Red Cross, (table 2).

	Cryo 250	SRBG-D
Volume	50 ml	20 ml
Tot. FVIII:C	± 250 IU	± 200 IU
Tot. prot.	1.015 g	0.888 g
Tot. fibrinogen	0.35 g	0.64 g
specific activity	± 0.24	± 0.23

table 2

In a much smaller volume of 20 ml only, it offers approximately the same amount of clotting activity at similar characteristics (s.a. 0.23 vs 0.24).
The smaller volume allows easy administration by syringe instead of iv-infusion, thereby improving the quality of life.

When the first cryoprecipitate is kept in the frozen state at −40°C there is no loss in Factor VIII:C or total protein recovery up to one year storage. However, longer storage under the same conditions shows rapid deterioration of the quality of the source cryoprecipitate, (table 3).

BATCH VARIATION: EFFECT OF STORAGE TIME

Time of storage	FVIII:C (IU/ML)	Protein (g/l)	Fibrinogen (g/l)
3 weeks n=2	5.3	25.8	20
11 months n=25	4.1 (± 1.3)	22.2 (± 4.7)	16.0 (± 3.9)
15 months n=20	1.5 (± 3.0)	10.9 (± 3.0)	7.8 (± 2.9)

table 3

CONCLUSION

Starting from routine citrated whole blood donations it is possible to produce with simple technology a small-pool freeze dried and virus inactivated cryoprecipitate with modest intermediate purity characteristics.
However, the procedure is based on minimum requirements for blood trans-

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fusion services of the second generation. The product is easy to handle and transport, does not require rigid storage conditions. It redissolved quickly in a small volume ready for injection, instead of the more complicated administration by iv-infusion.

Its normal Factor VIII molecular composition makes the products suitable for treatment of von Willebrand patients. The Factor VIII provides sufficient activity for treatment of Factor VIII deficient patients. The fibrinogen concentration of about 20 g/l allows the product to be used in emergency situations where fibrinogen consumption due to massive blood loss or DIC requires rapid suppletion to restore blood coagulation.

There are many situations in remote places, where access to blood transfusion is not guaranteed and the life of the patient therefore could be in jeopardy. Availability of this small pool, small volume freeze dried supercryoprecipitate therefore not only becomes of paramount importance to the haemophilic patient, but also to others, such as obstetric and trauma patients.

Although this contribution is modest, we believe that the concept might be of value for those developing countries or regions, where the second generation level of blood transfusion organisation has been reached or is about to be established. It supports the concept of component policy and optimal use of human blood and provides a safe and easy to handle semi-purified Factor VIII preparation readily accessable to haemophilia treatment, even under less optimal circumstances.

REFERENCES

1. The Haemophiliac in the eighties. Egli H and Inwood MJ eds. Haemostatis 1981; 10 (suppl. 1).
2. Ala FA. Comprehensive haemotherapy programme for developing countries. In: Das PC, Smit Sibinga CTh. Halle MR eds. Supportive therapy in haematology. Martinus Nijhoff, Publ. Boston/ Dordrecht/Lancaster 1985: 379–88.
3. Cash JD. Factor VIII concentrates. In: Ikkalen E and Nykänen A eds. Vammalan Kirjapaino OY. Vammalan 1975: 195–206.
4. Margolis J, Eisen M. Stabilising effect of amino acids on Factor VIII in lyophilized cryoprecipitate. Lancet 1984; ii: 1345.

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